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Supplementary Material

β_2-Adrenoreceptor is a regulator of the α-synuclein gene driving risk of Parkinson's disease

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1. Materials and Methods

Cell culture and drug library. Human neuroblastoma SK-N-MC cells were acquired from American Type Culture Collection (ATCC). Cells were cultured at 37°C in 5% CO_2 in DMEM/F12 supplemented with 10% fetal bovine serum (FBS) and 100 units per ml penicillin and streptomycin. The drug library was obtained from the Laboratory for Drug Discovery in Neurodegeneration (LDDN), Harvard NeuroDiscovery Center, Boston, MA. The drug library consisted of approximately 1,126 small molecules. About 586 of the compounds were from Prestwick Chemical's (Illkirch, France) library, a unique collection of mostly off-patent small organic molecules, 90% being marketed drugs and 10% bioactive alkaloids or related substances. About 400 compounds were from the SPECS collection (https://www.specs.net). 140 compounds generated in house by the LDDN were also included in the library (compounds labeled "LDN" in the Appendix). Compounds for the screening were stored as DMSO stocks. All drugs used for Confirmation Assay were purchased from Sigma (St. Louis, MO).

High throughput screening (HTS). *Screening Assay.* Approximately $2x10^4$ low passage SK-N-MC cells were seeded per well in 384-well cell culture plates for 24 hours. Compounds were then transferred into each well (final concentration 1μM) by using a BioMekFX (Beckman Coulter) equipped with a 384-channel pipetting head. After 48 hours in cell culture incubator, cells were washed once in cold phosphate-buffered saline (PBS) by using BioTek ELx405 select CW plate washer and then lysed at room temperature (22-24° C) with lysis buffer (Applied Biosystems) using the Cell-to-CT procedures according to the manufacturer's instructions (Applied Biosystems). After 5 min, lysis was terminated by incubation with stop solution (Applied Biosystems). 11 μL of each lysate were transferred to 384-well plates for RT in a total volume of 25 μl. 4 μL of RT reaction were then transferred to new 384-well plates for qPCR.

Using the Cell-to-CT kit (Applied Biosystems) we performed expression analysis directly from SK-N-MC neuroblastoma cells cultured in 384-well plates through *in situ* cDNA synthesis. In this miniaturized assay, high copy numbers of *SNCA* and of endogenous controls were detected as indicated by raw cycle threshold values of 25.11 ± 0.02 (mean CT value ± SEM) and 20.2 ± 0.02 for *SNCA* and Ubiquitin C (*UBC)*, respectively. Technical variation (using identical cDNA loaded in multiple wells) and biological variation (cDNA synthesized *in situ* in each of

384 wells of cultured SK-N-MC cells treated with DMSO) were low, with coefficients of variation of 1% and 5.2% respectively. At least ten DMSO-treated controls were included on each 384-well plate as negative external control and calibrator. An equal amount of cDNA derived from Universal Reference RNA was spotted on all plates as plate-to-plate control. Control reactions lacking template or reverse transcription were assayed for contamination with genomic DNA. Target and reference genes showed similar amplification efficiencies in a dilution series. For probe information refer Supplementary Table S2.

Threshold considerations. Genomic duplications of *SNCA* are sufficient to cause an autosomal dominant form of PD and risk variants associated with sporadic Parkinson's disease might lead to a subtle increase in *SNCA* expression (*1*)(*2*). *SNCA* duplications are predicted to cause a 1.5-fold increase in *SNCA* expression (three *SNCA* locus copies in patients carrying one duplicated allele vs. two copies in the reference genome). GWAS variants have been associated with increased susceptibility for common, sporadic PD and with small increases in *SNCA* expression *(4)* that over time may lead to detrimental consequences for dopaminergic neurons. We hypothesized that a reduction of *SNCA* expression by 20-35% may be sufficient for helping to address the increased *SNCA* expression of *SNCA* duplication carriers and in patients with common PD carrying the *SNCA* susceptibility allele. Based on these considerations we required a reduction of *SNCA* expression levels of >35% in the Screening Assay and >20% in the Replication and Confirmation Assays. Secondly, we selected the ubiquitin gene *UBC* as *reference gene* to control for RNA loading in the Screening Assay based on low variation in expression < 2% observed for this housekeeping gene in our preliminary studies of untreated and DMSO treated cells. Thirdly, we selected two additional, distinct housekeeping genes other than *UBC*, to control for RNA loading in the Replication and Confirmation Assays in order to identify compounds which robustly lower *SNCA* mRNA abundance independent of variation in housekeeping gene expression. Finally, we used a *P* value of ≤0.005 cutoff as an estimator of significance in the Confirmation Assay (adjusting the significance threshold for the number of ~14 drug classes tested in the Replication Assay (represented by 41 compounds forwarded from Screening to Replication), e.g. 0.05/~14 ≤0.005.

Screening assay: detailed threshold criteria. Compounds were forwarded from the Screening Assay into the Replication Assay, if they met the following threshold criteria: ***1***, Compound lowered *SNCA* mRNA expression levels by >35% (fold change < 0.65) compared to

DMSO treated cells (fold changes were rounded to two decimal points) *and **2***, CT values of the first housekeeping gene *UBC* were within one standard deviation of the mean (in order to eliminate outliers and possible cytotoxic compounds). 1,126 compounds were evaluated in the Screening Assay; 61 of 1,126 were associated with a reduction in relative *SNCA* mRNA abundance with fold change <0.65 compared to vehicle treated cells; 26 of these 61 compounds showed changes in the housekeeping gene *UBC* of greater than one standard deviation from the mean and were thus excluded; the remaining 35 of 61 compounds were forwarded to the Replication Assay. Furthermore, six related drugs were added ("hit expansion").

Replication assay and detailed threshold criteria. For each compound eight wells of SK-N-MC cells were treated with drug and compared to eight wells treated with DMSO alone. The Cell-to-CT procedure was employed using two new reference genes, ribosomal protein L13 (*RPL13)* and glucuronidase beta (*GUSB*) (instead of *UBC)* to control for input RNA. Compounds were forwarded from the Replication to the Confirmation Assay, if they met the following threshold criteria: ***1***, A compound reduced relative *SNCA* mRNA abundance using the second housekeeping gene *RPL13* compared to vehicle treated control cells (as indicated by fold change < 1.0); *and **2***, reduced relative *SNCA* mRNA abundance by 20% or more (fold change <0.80) compared to vehicle treated control cells using the third housekeeping gene *GUSB*. 10 compounds met these criteria and were forwarded from the Replication into the Confirmation Assay.

Confirmation assay and detailed threshold criteria. The ten forwarded compounds were used in the Confirmation Assay. For each compound ten wells of SK-N-MC cells were treated with drug and compared to ten wells treated with DMSO alone. RNA was extracted using TRIzol® following the manufacturers protocol (Life Technologies). Freshly ordered compounds were used. Compounds were forwarded from the Confirmation to the ELISA Assay (see below), if they met they following significance criteria: ***1***, Compound reduced relative *SNCA* mRNA abundance by at least 20% (fold change <0.8) compared to vehicle treated controls and ***2***, with a p value ≤ 0.005. Six compounds met these criteria and were forwarded from the Confirmation Assay to the *ELISA Assay* stage (see below).

NanoString assay performance. Probes were designed according to the manufacturer's design principles, including screening for inter- and intra-reporter and capture probe interactions, and

selection for probes with optimal melting temperatures. For target sequences information for NanoString probes refer Supplementary Table S2. Direct counts of the target RNAs were measured in 125 ng of RNA by digital expression analysis based on NanoString technology (without reverse transcription into cDNA). Probes for the target and control RNA were multiplexed and assayed according to the manufacturer's protocol on the nCounter Digital Analyzer. The laboratory running the assay was blinded to the diagnosis. No-template (negative) controls containing water substituted for template were run and no signal was detected.

Cell viability assay for neuroblastoma cells. CellTiter-Glo® Assay was used for measuring the cell viability in SK-NM-C cells. 100 μL CellTiter-Glo reagent (Promega) was added to 100 μL of cell culture medium per well in 96 well plates. Plates were agitated for 2 min and incubated for 10 min at room temperature (22–24^{o} C) before luminescence was measured.

Cortical neuronal cultures. Primary cortical cultures were generated from E18 Sprague-Dawley rat embryos. The cortical region was dissected out in Hank's Balanced Salt Solution buffered with Hepes and dissociated with 0.125% trypsin (Invitrogen) for 17 min at 37 °C, followed by trituration. Dissociated cells were plated at a density of 5×10^5 cells/well or 2.5×10^5 cells/well in 12-well or 24-well plates, respectively precoated with poly-D lysine (100 μg/mL). The cells were cultured in Neurobasal medium with B-27 supplement (Invitrogen) and glutamax and gentamycin. Half of the medium was changed every 4 days. Cortical neurons were treated with β_2AR agonists 10 days post plating. They were then harvested 48 hours post-treatment for mRNA extraction and 72 hours post-treatment for protein extraction.

Hippocampal neuronal cultures. Primary hippocampal cultures were generated from day 0 neonatal wild type and β_2AR KO mice. The hippocampal neurons were plated on poly-D lysine (100 μg/mL) precoated plates and cultured in Neurobasal media with B-27 supplement and L-glutamine for 14 days. Post 14 days after plating the cells were harvested for mRNA and protein extraction.

Human pluripotent stem cell culture. The derivation and culture of the WIBR-IPS-αSyn^{TRPL} line has been described previously (*27*).

Human neural induction by embryoid body (EB) formation. To initiate differentiation, iPSC colonies were pretreated for 30-60 min with 5 μM Y-27632, a ROCK inhibitor (Calbiochem). They were single cell-dissociated after 5-10 minute exposure to accutase (StemProAccutase; Life Technologies) and then re-suspended in neural base (NB) medium, which is DMEM/F12 (Gibco/Life Technologies) supplemented with 0.5% N2 and 1% B27 (Life Technologies). Cells were plated in AggreWell 800 microwells (StemCell Technologies; priming and plating per manufacturer's protocol; $2.4x10^6$ cells were well) in NB medium supplemented with dual SMAD inhibitors (recombinant human Noggin (R&D Systems) at 200 ng/mL and 10 μM SB431542 (Tocris Bioscience), as well as 5 μM Y-27632. Noggin and SB431542 remained in the medium at these concentrations throughout the neural differentiation protocol.

On day 1 medium was half-changed. By day 2, well-formed neuralized EBs (NEBs) were typically observed in the AggreWells and transferred to Petri dishes (4 AggreWell wells/ Petri dish) overnight, in NB medium. On day 4, NEBs were transferred to a dish coated with growth factor-reduced Matrigel (1:30 in DMEM: F12; BD Biosciences) for attachment. Y-27632 was omitted from this day until day 10. From day 5 to day 10, attached NEBs were additionally exposed to 20 ng/mL FGF2 (R&D Systems) and recombinant human Dkk1 at 200 ng/mL (R&D Systems). On day 10, neural rosettes were dissected (P20 pipette tip), incubated in accutase supplemented with DnaseI (Sigma Aldrich) for 10 min at 37^oC, and gently dissociated to small cellular clumps and single cells. After washing, the rosettes were re-plated on plastic dishes pre-coated with poly-L-ornithine and laminin (BD Biocoat) at high density ($200,000/cm^2$) in neural progenitor cell (NPC) medium, which is NB medium supplemented with 20 ng/mL FGF2 (Life Technologies), supplemented overnight with 10 μM Y-27632. Typically, one Aggrewell 800 well provided sufficient NPCs for one to two 6-well plates at passage 0.

Thereafter, the surviving NPCs proliferated. Medium was changed daily. NPCs could be passaged up to 10 times before neural differentiation, and could be successfully freeze/thawed at early passage (p1 to p5) without compromising differentiation potential. The freezing medium used was NPC medium with 10% FBS (Hyclone).

Human cortical neural differentiation. To begin neural differentiation, NPCs were dissociated with accutase and re-plated on matrigel-coated T75 flasks (CytoOne). The next day medium was

fully changed to Neural Differentiation (ND) medium, which is NB medium supplemented with recombinant human BDNF and GNDF (both at 10 ng/mL; R&D Systems) and dibutyryl cyclic AMP (500 μM; Sigma), and without FGF-2. Thereafter, media was half-changed every other day. On day 7-9, differentiating neurons were gently dissociated to single cell suspension and then resuspended in pre-chilled Hank's balanced salt solution (HBSS; Gibco / Life Technologies) supplemented with 0.1% bovine serum albumin (Gibco / Life Technologies). After a wash step, cells were plated on 6- or 24- well plates pre-coated with poly-ornithine and laminin (BD Biocoat). For maximum survival, 5 μm Y-27632 was used in the initial plating medium and cells were plated at high density (500,000-1x10^6 cells/cm^2). Medium was half-changed every 3 days for up to 8 weeks. After 8 weeks, cells were exposed to compound for 3 days, with a complete medium change every other day.

Pharmacokinetics mouse study. The clenbuterol pharmacokinetic (PK) study was done in wild-type C57BL/6J male mice by ChemPartner Co., LTD in Shanghai. The study was done with 4 groups of four mice; one control group (i.p. vehicle at 10 ml/kg) and three treatment groups with clenbuterol administered via intraperitoneal injection at dosages of 1, 5 and 10 mg/kg. Blood samples were collected at 24 hours from the four mice per group. Mice were sacrificed following each blood collection and brains were removed and snap frozen. Substantia nigra was utilized for *SNCA* mRNA analysis. Plasma and brain samples were analyzed by LC-MS/MS for compound concentration in each of the four treatment groups.

Placebo-controlled trial in wild-type mice. Twenty C57BL/6J male mice (8 weeks old) (Jackson Laboratory, Bar Harbor ME) were used for the placebo-controlled trial. Mice were exposed to a 12-hour light and 12-hour dark cycle, maintained at a constant temperature of 22°C, and were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Clenbuterol was purchased from Sigma (St. Louis, MO). Clenbuterol (10 mg/kg, injection volume 10 ml/kg) dissolved in saline was administered to mice (*N*=10) intraperitoneally. Saline was administered as a vehicle treatment (*N*=10). Mice were euthanized by CO_2 24 hours post-injection. Brains were removed and snap frozen immediately in dry ice. Further dissections were performed on the frozen tissue to obtain substantia nigra regions. Tissue was then stored at -80°C until mRNA or protein extraction was performed.

Controlled trial in the MPTP mouse model. Male C57bl/6 mice (8–10 weeks old) were analyzed. N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP-HCl; 20 mg/kg, measured as free base; Sigma, St. Louis, MO) was administered to mice intraperitoneally once per day for five consecutive days similar to described previously *(26,36)*. Eight MPTP-treated mice assigned to the clenbuterol treatment group received one 10 mg/kg i.p. injection of clenbuterol (two days prior to MPTP treatment start) and then clenbuterol via oral gavage (10 mg/kg) daily for two weeks (starting on the day of the first MPTP treatment). Another eight MPTP-treated mice assigned to the vehicle treatment group received only oral drinking water (without clenbuterol). Additional control mice received i.p. injections of equivalent volumes of saline (0.9%) instead of MPTP intraperitoneally once per day for 5 consecutive days; of these eight received drinking water alone (without clenbuterol) and another eight received oral clenbuterol. Mice were sacrificed 14 days after initial MPTP/saline i.p. treatment.

After overnight postfixation in 4% paraformaldehyde, brains were cryoprotected in 30% sucrose before cryosectioning into 40 μm (used for stereology) free-floating sections, obtained as described previously *(36)*. Immunostaining was performed as described previously *(36)* using mouse anti-tyrosine hydroxylase (Abcam; 1:700) and diaminobenzidine (DAB). TH+ cells were analyzed by sterological estimates as described previously *(36)*. Briefly, the total number of TH+ neurons in the SNc was calculated by optical fractionation using Stereo Investigator (version 6; MicroBrightField, Williston, VT). 40 μm of brain sections were examined from bregma −2.54 to −3.88 of the SNc. For each brain, six coronal sections were examined using a 100X lens. Total number of TH+ neurons was determined using the optical fractionator. Adjacent tissue was also stained using cresyl violet (Sigma, 0.01 % for 5 min) to confirm cell loss as assessed by TH analysis.

siRNA transfection. SK-N-MC cells grown in 6-well dishes at 40% confluence were transfected with SMARTpool: ON-TARGET plus *ADRB2* siRNA (cat no. L-005426-01-0005, GE Healthcare Dharmacon Inc.) and ON-TARGET plus Non-targeting Pool siRNA (cat no. D-001810-10-05, GE Healthcare Dharmacon Inc.). The required amount of SMART-pool target siRNA (Dharmacon) and 5 μL of Lipofectamine RNAiMAX (Invitrogen) were each diluted into a final volume of 250 μL in Opti-MEM (GIBCO), combined, gently mixed, and incubated at

room temperature for 20 min. 500 μL of this transfection solution was overlaid onto cells at a final concentration of 80 nM siRNA. Transfection of SK-N-MC cells with ON-TARGET plus Non-targeting Pool (Dharmacon, with no significant homology to any known gene sequences from mouse, rat, or human) served as a negative control. Cells were treated with clenbuterol (10 μM) 24 hours after transfection and lysed after 48 hours of treatment by TRIzol reagent for mRNA and 1x PBS + 0.5% NP-40 for protein extraction.

RNA extraction and qPCR. Total RNA from flash-frozen mice brain tissue, rodent primary cells, human neuroblastoma cells and iPSC-derived neuronal cells was isolated using TRIzol® (Life Technologies) following the manufacturer's protocol. RNA concentration was measured using NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). 2 μg of total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit from Applied Biosystems. Quantitative PCR was performed using TaqMan Universal PCR Master Mix (Applied Biosystems) on an ABI 7900HT instrument (Applied Biosystems) as described previously (*31*). For mouse brain tissue and rat primary cortical neuron samples, rodent-specific TaqMan Assay on-demand primers and probes were used to assay the relative abundance of target genes, and rodent *Rpl13* was used as a reference gene to normalize for RNA loading. Similar results were obtained when *Actb* or *Ubc* were used as controls for input RNA. For human neuroblastoma and iPSC-derived neuron samples, human TaqMan Assay on-demand probes were used to assay the relative abundance of target genes, and the geometric mean of the three human reference genes *RPL13, GUSB* and *UBC* was used to normalize for RNA loading. Expression values were analyzed using the comparative threshold cycle method (*31*). Primers used for qPCR are listed in Supplementary Table S2.

Protein extraction. Protein lysis buffer containing 320 mM sucrose, 5 mM NaF, 1 mM Na_3VO_4, 10 mM Tris (pH 7.4), 1 mM EGTA, and 1 mM EDTA was used for mice brain tissue lysis. 100 μL of cold extraction buffer was added to each sample and homogenized in a dounce homogenizer. After incubating on ice for 10 minutes post-homogenization, samples were centrifuged at 10,000 rpm at 4ºC for 10 minutes. Supernatant was transferred to new tubes and centrifuged again at 10,000 rpm at 4ºC for additional 10 minutes. The final supernatant was transferred to new tubes and protein estimation was performed by ELISA. Total protein from

rodent primary cells, human neuroblastoma cells and iPSC-derived neuronal cells was extracted using lysis buffer containing 1x PBS and 0.5% NP-40. Cells were washed once with cold PBS and lysed by adding cold lysis buffer. The cells were collected by scraping, then incubated for 30 minutes by rotating at 4ºC. The samples were centrifuged at 14,000 rpm at 4ºC for 10 minutes and supernatant was transferred to new tubes. Total protein concentrations were determined by BCA assay (Thermo Scientific) according to the manufacturer's instructions. Total α-synuclein was quantified using an α-synuclein-specific sandwich ELISA.

Antibodies. Antibodies used were anti-α-synuclein, clone 2F12 (*11*) (EMD Millipore, 1:1000 for Western blotting of endogenous α-synuclein, also used for ELISA), anti-α-synuclein, clone SOY1 (*11*) (EMD Millipore, used for ELISA), monoclonal anti-α-Synuclein Syn-1 (Clone 42, Becton-Dickinson; used for ELISA), anti-α-synuclein, clone 1H9 (courtesy Dr. Selkoe lab; used for ELISA), anti-α-synuclein, C-20 (Santa Cruz Biotechnology, 1:1000 used for Western blotting), anti-Histone 3 (acetyl K27) (abcam, 1:1000 for Western blotting, also used for ChIP assay) and anti-α-tubulin (Cell Signaling Technology, 1:1000 for western blotting).

α-synuclein-specific ELISAs. An α-synuclein-specific Meso Scale Discovery (MSD) ELISA assay (described in Ref. (*11*)) was utilized for endogenous α-synuclein protein measurement. 96-well Multi-Array Standard Bind plates (MSD, Rockville, MD) were coated with the capture antibody 2F12 (*11*) diluted to 6.7 ng/µl in phosphate-buffered saline in 30 µl volumes/well and incubated at 4 °C overnight. Following emptying of the wells, plates were blocked for 1 hour at room temperature in blocking buffer (5% MSD Blocker A; TBS-T). After 3 washes with TBS-T, samples diluted in TBS-T with 1% MSD Blocker A and 0.5% NP-40 were loaded and incubated at 4 °C overnight. After samples were emptied and plate washed 3 times with TBS-T, sulfo-tagged SOY1 mAb (detection Ab) (generated using Sulfo-Tag-NHS-Ester (MSD) diluted in blocking buffer (6.7 ng/µl), was added to the plate (30 µl volumes/well) and shaken for 1 hour at room temperature. Following 3 washes, MSD Read (2X, diluted in ultrapure water) buffer was added and the plates were immediately measured using a MSD Sector 2400 imager. For confirmatory studies, an second α-synuclein-specific ELISA implementation was used employing a different, extensively published anti-α-synuclein detection antibody (Syn-1 mAb) and a different anti-α-synuclein capture antibody (1H9, courtesy Dr. Selkoe).

Immunoblotting. For western blot analysis, protein samples (20 µg) were run on a 4-20% gradient SDS–polyacrylamide gel (Biorad) and transferred onto a polyvinylidene fluoride (PVDF) membrane filter (0.2 µm; Biorad) using an electroblot apparatus (Bio-Rad, Hercules, CA) at 100V for 1h in transfer buffer (25 mM Tris–HCl, 192 mM glycine, 0.1% SDS, 20% (v/v) methanol). The membrane was incubated in blocking solution (50 mM Tris–HCl, 200 mM NaCl, 1 mM $MgCl_2$, pH 7.4) containing 5% non-fat dry milk powder for 1 hour at room temperature. The membrane was processed through sequential incubations with primary antibody overnight at 4^oC followed by incubation with horseradish peroxidase-conjugated species specific secondary antibody at 1:250 dilutions (Thermo Scientific Pierce). Immunoreactive proteins on the membrane were visualized using the SuperSignal™ West Pico Chemiluminescent kit (Thermo Scientific Pierce). Scanned western blots were analyzed using ImageJ software, version 1.47. Pictures were inverted and usually the background signal from an empty lane was subtracted to obtain specific signals for each lane.

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation (ChIP) assays were performed using the Simple ChIP Enzymatic Chromatin IP kit protocol as recommended by the manufacturer (Cell Signaling Technology) with slight modifications. Briefly, ChIPs were performed on asynchronously growing SK-N-MC cells treated with clenbuterol (10 µM), valproic acid (100 µM) or propranolol (100 µM). Cross-linking was carried out with 1% formaldehyde for 10 min at room temperature. Cross-linking was subsequently quenched by adding glycine to a final concentration of 1x for 5 min. Cells were collected and washed twice with PBS, then resuspended in 2 mL of lysis buffer (as described in the protocol). After 10 min on ice, cells were treated with micrococcal nuclease and then sonicated to obtain DNA fragments of ~500 bp as determined by agarose gel electrophoresis with ethidium bromide staining. Protein-DNA complexes were isolated by centrifugation at 10,000 rpm for 10 min. Supernatants with protein-DNA complexes were incubated overnight with rabbit polyclonal antibody directed against acetylated H3K27 (Abcam). Normal rabbit IgG was used as a negative control and Histone H3 rabbit mAb (ChIP formulated) as positive control. 2% of sample was saved as input. Antibody-protein-DNA complexes were further incubated for 2 hours with 30 µl of ChIP-grade Protein G magnetic beads to isolate antibody bound fractions of chromatin. Immuno-complexes

were washed with the buffers provided and as described in the kit. Protein-DNA complexes were eluted and cross-links of pull down fractions and inputs (2% of total IP fraction) were reversed by 2 hours incubation in Proteinase K and 5 M NaCl at 65^{o}C. DNA was then extracted, purified, precipitated, and resuspended in TE for qPCR. 2 µl of immunoprecipitated DNA was analyzed by qPCR using a *Power* SYBR Green PCR Master mix (Applied Biosystems by Life technologies) on an ABI 7900HT instrument (Applied Biosystems) with the following temperature profile: 3 min of enzyme activation at 95°C followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Primers used for qPCR are listed in Supplementary Table S2. Sample from three independent immunoprecipitation assays were analyzed.

Mitochondrial superoxide and viability assay for iPSC-derived neuronal precursor cells (NPC). Patient-derived NPCs carrying the familial *SNCA* triplication were generated as described previously *(28)*. NPCs were seeded at 3.5x10^4 cells/mm^2 in 96-well plates (Greiner Cellcoat µClear) for HTS plate reader analysis (Polarstar Omega, BMG). NPCs were cultured in high glucose growth medium.

For the Plate Reader HTS, each experiment was conducted with 6 replicates per cell line and treatment regimen. NPCs were treated with or without 20 µM clenbuterol for four days and challenged with 20 µM rotenone during the last 18 hours. Signals from individual wells were acquired with integration of fluorescence signals over 1 second duration by bottom read in orbital mode (8-spot measurements with 2 mm radius). Data analysis was performed using BMG Mars software. For data analysis of individual experiments, the mean ± SD was plotted using GraphPad7. Non-directional Student's t-test was used for direct statistical comparisons. For multiple comparisons, 2-way and multivariant ANOVA were used. Where significant *F*-values were obtained, pairwise comparisons were made using Tukey's post hoc analysis. Differences were considered statistically significant at $p \leq 0.05$.

For the MitoSox assay for the detection of mitochondria-associated superoxide levels, adherent NPCs in 96-well plates were incubated with 2 µM MitoSOX™ (Ex./Em. 510/580nm) and MitoTracker® Green (485/520 nm) (both Life Technologies) in high glucose without phenol red (Life Technologies), containing supplements without antioxidants (Life Technologies) for 15 min at 37°C in the dark. Cells were then washed twice with medium (also containing 1µM

Hoechst 33342). Fluorescence was detected by sequential readings, and MitoSOX™ signals were normalized to mitochondrial content (Mitotracker®) and cell number (Hoechst).

For the Resazurin assay for reduction potential by NADPH levels, NPCs were prepared as above and then loaded with 3 μM C12-Resazurin (Ex/Em: 563nm/587nm) (Life Technologies) in medium (see above), for 30 min at 37°C. Loading solution was then removed and cells were washed twice with full growth medium containing 1 μM Hoechst 33342. Relative C12-Resazurin fluorescence intensities were normalized to Hoechst 33342 fluorescence.

Statistical analyses of *SNCA* expression. Two-tailed Student's t-tests were used for two-group comparisons as indicated in the figure legends; one-way or two-way ANOVAs with Tukey's post-hoc test were used for analyses involving multiple groups as indicated in the figure legends. *P*-values equal to or lower than 0.05 were considered statistically significant.

Analyses using the Norwegian Prescription Database

Study population: We included the entire population of Norway alive on 01.01.2004 by linking the Norwegian National Registry (NNR) with the Norwegian Prescription Database (NorPD) using the unique national identification number. The NNR contains information on all residents in Norway, time of immigration, emigration and death. This information was further linked to the Norwegian National Education Database (NNED) to assess level of education of all participants.

NorPD: This registry contains complete information on all prescribed drugs dispensed at pharmacies to individuals since 2004. Each prescription includes the national identification number, which makes it possible to generate a precise chronological prescription record for individuals over time, and to link this information to other registries. Drugs are classified according to the Anatomical Therapeutic Chemical classification and doses are reported as defined daily dose (DDD). If the national insurance scheme covers the cost of the drug, the prescription also contains the diagnosis the drug was prescribed for (from 2008: ICD-10 and ICPC; before 2008: local diagnostic code).

Definition of outcome: We defined an incident case of PD as an individual who had received at least 365 DDD of levodopa (N04BA), which corresponds to one year of use at the average maintenance dose per day of the drug, and who was given a diagnosis of PD (ICD-10:

G20) as the reason for the prescription. The first year of prescription of any antiparkinson drug (N04) was considered year of onset of PD. We used the year 2004 as a washout-period to only include incident cases. By using this definition, we found a total of 10,070 individuals with PD, of whom 4,634 were incident cases in the period 2005-2014. This gave a mean annual incidence rate of 10.3 per 100,000, which is very similar to what was reported in the most recent large clinical epidemiological study of PD in Norway (12.6 per 100,000) (*17*). The mean age of first prescription of antiparkinson drug was 71.3 years with 58.3% males compared to 69.4 years at onset and 57% males in the clinical study.

Exposure definition: There were 619,863 users of salbutamol (ATC: R03AC02 (inhalable) and R03CC02 (systemic)) and 273,356 users of inhaled corticosteroids (ATC: R03BA) during follow-up, while there were 295,387 users of salbutamol (ATC: R03AC02 (inhalable) and R03CC02 (systemic) during 2004-2007. Participants were categorized as exposed at time of first prescription of the relevant drug. In the same period, there were 63,210 users of propranolol (ATC: C07AA05), of whom 33,864 were prescribed the drug because of non-neurological indications covered by the national insurance scheme. Specifically, this included the ICD-10 codes -22, G43, I10, I11, I12, I13, I15, I20, I21, I22, I47, I48, I49 and Z94, the ICPC codes -22, -51, K74, K75, K78, K79, K80, K86, K87 and K89, and the local diagnostic code 012 (Morbus Cardiovascularis). To further limit the chance of reverse causality, we only included users who had been prescribed at least 365 DDD or more of propranolol. This included 14,794 users and these individuals were categorized as exposed at time of prescription of propranolol where 365 DDD was exceeded. For the period 2004-2007, there were 9,339 users of propranolol fulfilling the same criteria.

Statistical analysis: The level of education was categorized into primary education, secondary education, undergraduate education or graduate education. Age at study start was categorized in 5 years intervals. We calculated risk estimates (hazard ratio) with 95% confidence intervals using a Cox proportional hazard regression model, which included the exposure of interest (salbutamol, propranolol or corticosteroids) as a time-dependent covariate adjusting for sex, age and level of education. Outcome was PD as defined above at time of first prescription of an antiparkinson drug. Participants not developing PD were censored at end of follow-up (31.12. 2014), at date of death or date of emigration out of Norway, whichever occurred first. To test for dose-response effects, we categorized participants according to the cumulative number of DDD

in 2004-2007 and estimated the RR of developing PD in 2008-2014. Those who developed PD, died or moved out of Norway before 2008 were excluded from these analyses (n=189,584). The hazard ratios were interpreted as rate ratios.

Ethical approval: The study was approved by the Regional Committee for Medical and Health Research Ethics (REK Vest).

Information on data access to NorPD can be found at http://norpd.no; NNR at http://www.skatteetaten.no/en/person/National-Registry/This-is-the-National-Registry/; NNED at http://www.ssb.no/en/omssb/tjenester-og-verktoy/data-til-forskning/utdanning. Access to data and linkage of registries require ethics approval and permission from the individual registries.

Post-mortem brain CAGE methods

Sample preparation and RNA extraction. Postmortem substantia nigra tissue samples were obtained from University of Maryland, University of Washington, and McLean Hospital (Belmont, MA). Four human post-mortem samples (healthy controls) were utilized for CAGE. 5 µg of total RNA was exacted from each sample using the RNeasy RNA Kit (Qiagen) with an RNA integrity number (RIN) > 6. Postmortem brains were collected under local IRB approval. Use of postmortem samples for expression analysis was approved by the IRB of Brigham & Women's Hospital.

Library preparation. Libraries were constructed using a published CAGEseq protocol adapted for next-generation sequencing *(37)*. Briefly, complementary DNA (cDNA) was synthesized from total RNA using random primers, and this process was carried out at high temperature in the presence of trehalose and sorbitol to extend cDNA synthesis through GC-rich regions in 5′ untranslated regions. The 5′ ends of messenger RNA within RNA-DNA hybrids were selected by the cap-trapper method and ligated to a linker so that an EcoP15I recognition site was placed adjacent to the start of the cDNA, corresponding to the 5′ end of the original messenger RNA. This linker was used to prime second-strand cDNA synthesis. Subsequent EcoP15I digestion released the 27-base pair (bp) CAGEseq reads. After ligation of a second linker, CAGEseq tags were polymerase chain reaction amplified, purified, and sequenced on the HiSeq 2000 (Illumina) using standard protocol for 50 bp single end runs.

CAGE expression analysis. CAGEseq data were filtered for CAGEseq artifacts using

TagDust (version 1.12), removal of reads mapping to known ribosomal RNA genes and low quality reads, mapping to the human genome (hg19) using Burrows-Wheeler Aligner (version 0.5.9) for short reads. Reads mapping to autosomes were used to minimize gender and normalization biases for subsequent analysis. Normalization was done based on the amount of reads per million sequence reads.

2. Supplementary Text

Supplementary Text S1. β2AR activation selectively modulated the expression of *SNCA* without adversely affecting neuronal cell viability or housekeeping gene expression (fig. S3). To determine whether the effect of β2AR agonists on *SNCA* expression is selective or part of a general effect, we surveyed a ribosomal gene, *RPL13*; the housekeeping gene, *GUSB*; as well as a structural neuronal gene, neurofilament light chain, *NEFL*. No material transcriptional off-target effects were observed in these gene expression assays (fig. S3).

Supplementary Text S2. β2AR agonists lowered *SNCA* expression in a dose- and time-dependent manner. Increasing concentrations of clenbuterol (5, 10, 20 μM) were associated with a decrease in α-synuclein mRNA (Fig. 1E) and protein (Fig. 1F) levels in SK-N-MC cells. The two β2AR agonists, metaproterenol and salbutamol, similarly lowered *SNCA* mRNA expression in a dose-dependent manner (fig. S6). We treated SK-N-MC cells with clenbuterol (at a concentration of 10 μM) for two, three, or four days (fig. S5) and observed a time-dependent effect on α-synuclein regulation. First, *SNCA* mRNA levels progressively declined upon β2AR agonist exposure compared to controls (fig. S5a). Second, with a lag time of about two-three days, α-synuclein protein levels progressively declined at day four of agonist exposure (fig. S5b) consistent with the long half-life of α-synuclein protein. *SNCA* mRNA and α-synuclein protein expression remained low after prolonged clenbuterol treatment (fig. S12).

Supplemental Text S3. *SNCA* transcription appears to be finely regulated through a classical promoter spanning the non-protein-coding exon 1 and intron 1 sequences at the 5' end of the *SNCA* locus and through two enhancers in the long intron 4. We clarified the endogenous *SNCA* promoter and putative enhancer sites using Cap analysis gene expression (CAGE) in human PD-relevant substantia nigra (this study), and by examining human brain RNA-seq data from GTEx (*34*), and histone and transcription factor ChIP-seq data from ENCODE (*35*). This integrative genomics view of the *SNCA* locus clarified the promoter of *SNCA* (Fig. 3A, site 1) on the basis of human substantia nigra CAGE peaks, human brain RNA-seq reads, and histone markings (high H3K4Me3 and low H3K4Me1 (Fig. 3A). ENCODE ChIP-seq data revealed that the *SNCA* promoter is directly occupied by a diverse set of transcription

factors in multiple cell types (*34*) including TATA-box binding protein (TBP), polymerase (RNA) II polypeptide A (POLR2A), and GATA family transcription factors, which regulate *SNCA* transcription (*5*). Two putative, active enhancers in intron 4 showed enrichment of marks of active enhancers with low H3K4Me3 and high H3K4Me1 signals (Fig. 3A, sites 2 and 3). Moreover, the enhancer hallmark, histone acetyltrasferase P300 (EP300), which functions as a histone acetyl transferase to regulate transcription via chromatin remodeling, was enriched in both intronic enhancer loci (Fig. 3A). Additionally, H3K27ac signals (indicative of active enhancer elements) were observed at the promoter and the two enhancers regions. Since β2AR-stimulation has been implicated in regulating WNK4 transcription via histone acetylation in renal cells (*14*), we hypothesized that β2AR activation may regulate *SNCA* transcription through an analogous mechanism.

Supplemental Text S4. Definition of PD patients in the epidemiology studies. Individuals who had been prescribed levodopa specifically for PD were defined as PD patients. Further, we restricted the analyses to individuals who had used levodopa more than one year, to avoid the inclusion of individuals with a less certain diagnosis. This resulted in a yearly incidence rate similar to a recent clinical incidence study in Norway (*17*).

Supplemental Text S5. Clenbuterol treatment can protect against MPTP-induced degeneration of tyrosine hydroxylase (TH)-positive neurons in mice. In addition to α-synuclein, chemicals such as N-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) (*21, 22*) and rotenone (*23, 24*) are implicated in the mechanism of sporadic PD. These chemicals inhibit the flow of electrons through complex I of the electron transport chain and foster buildup of superoxide and other reactive oxygen species particularly in dopamine neurons (*22, 25*). We tested whether clenbuterol treatment can protect against MPTP-induced degeneration of tyrosine hydroxylase (TH)-positive neurons in the substantia nigra pars compacta (SNpc) of a mouse model of PD (*22*). Mice were assigned to a treatment arm (n = 8) that received clenbuterol (10 mg/kg/day) orally by gavage for two weeks starting two days before the first MPTP injection or a control arm (n = 8). All mice received daily injections of MPTP (20 mg/kg; i.p.) for five consecutive days according to well-established protocols (*26, 36*). Two weeks following the last MPTP injection, mice were euthanized for stereological analysis. MPTP-injected mice displayed

a 40% loss of tyrosine hydroxylase-positive (TH+) neurons in the SNc compared to saline-injected controls. Clenbuterol treatment abrogated the MPTP-induced loss of TH+ neurons (Fig. 4C, D). Importantly, we evaluated cresyl violet (CV) stained nigral cell counts in drug treated and untreated mice. Consistently, clenbuterol treatment blocked the loss of cresyl-violet stained cells in the SNpc (Fig. 4E, fig. S10). Thus, clenbuterol appeared to be protective against MPTP-induced loss of TH positive nigral cells in this mouse model of PD.

3. Supplementary Figures

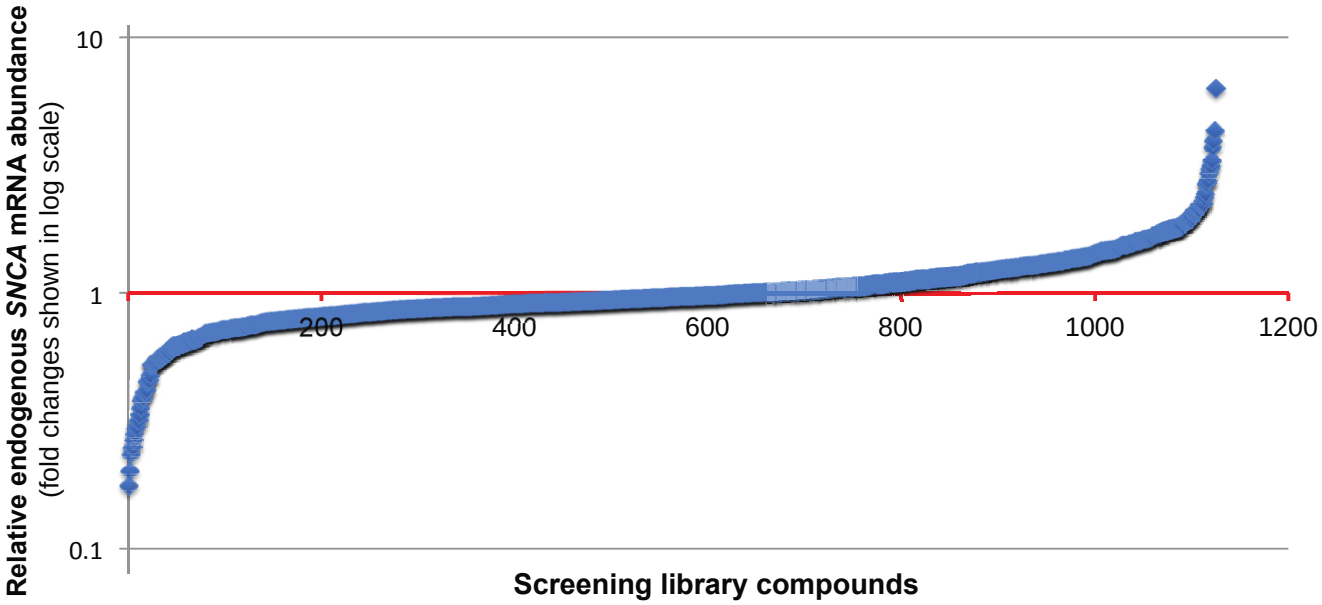

Figure S1. "Campaign View" of compounds screened in the cell-based, endogenous *SNCA* mRNA Screening Assay. For each compound the corresponding fold-changes in relative *SNCA* mRNA abundance (y-axis, $\log_{10}$ scale) observed in the drug-treated human neuroblastoma cells compared to DMSO-treated human neuroblastoma cells are shown (the housekeeping gene *UBC* was used to control for input RNA). 1,126 compounds representing FDA-approved drugs and a diverse set of natural products, vitamins, health supplements, and alkaloids were screened and are shown on the x-axis (sorted by ascending fold-change; except for one extreme outlier, which was omitted from the figure). 35 compounds met the Screening Assay threshold criteria and lowered *SNCA* expression by more than 35% in the screening stage, including the selective β_2-adrenoreceptor agonist metaproterenol. Six additional compounds, including the two selective β_2AR agonists, clenbuterol and salbutamol (that did not meet threshold criteria in the Screening Assay) were added at the replication stage ("hit expansion").

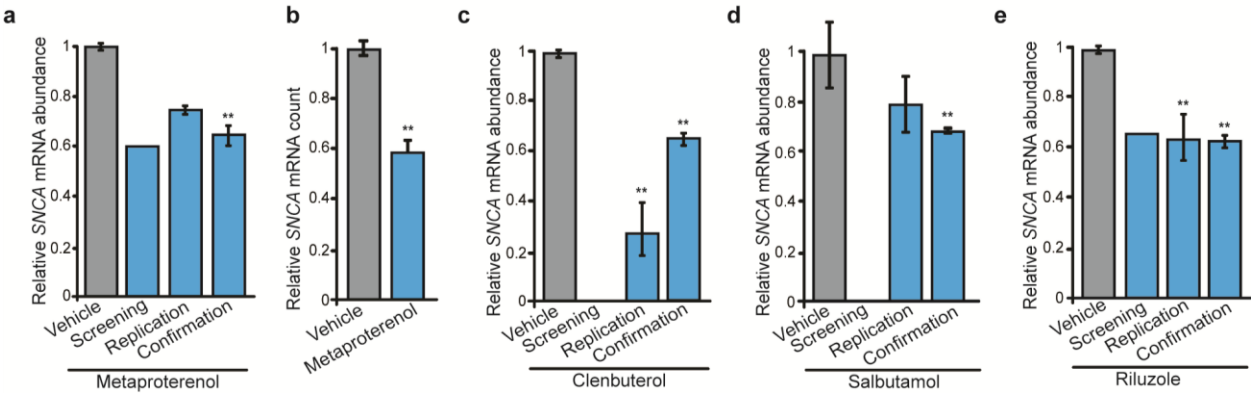

Figure S2. High throughput screen identified β_2AR agonists lowering endogenous *SNCA* mRNA expression. a) Metaproterenol consistently lowered *SNCA* mRNA abundance at the screening (1 μM for 48 hours), replication (1 μM for 48 hours) and confirmation (1 μM for 48 hours) stages compared to vehicle control ($P< 0.005$ in the confirmation stage). b) An independent digital gene expression platform (Nanostring) confirmed the effect of metaproterenol on *SNCA* mRNA repression. c-d) Clenbuterol and salbutamol lowered relative *SNCA* mRNA abundance in the replication (1 μM for 48 hours) and confirmation stage (1 μM for 48 hours). Note, that these two compounds failed formal threshold criteria in the screening stage; thus no bar graph is shown for the screening stage. However, because of their relation to metaproterenol these drugs were re-added to the replication stage ("hit expansion"). e) Riluzole treatment lowered relative *SNCA* mRNA abundance at the screening, replication, and confirmation stage (1 μM for 48 hours for each experiment, respectively). SK-N-MC cells were used in these experiments. $n = 8$ and $n = 10$, for drug treated or vehicle-treated cells in the replication and confirmation assays, respectively; **, indicates $P<0.005$, two-tailed Student's t-test.

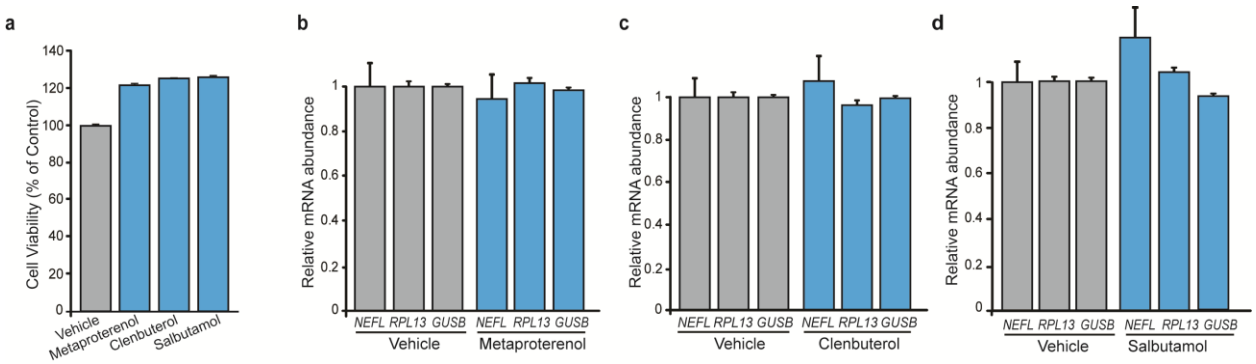

Figure S3. β_2AR activation selectively modulates the expression of *SNCA* without adversely affecting neuronal viability. a) Treatment with β_2AR agonists, metaproterenol (10 μM), clenbuterol (10 μM), or salbutamol (5 μM) does not alter cell viability as measured by cellular ATP levels. b-c) Treatment with metaproterenol (10 μM), clenbuterol (10 μM), or salbutamol (5 μM) for 48 hours compared to vehicle, respectively, did not affect relative mRNA abundance of *NEFL*, *RPL13* and *GUSB* as measured by qPCR in SK-N-MC cells. *n* = 6-8, means ± SEM are shown.

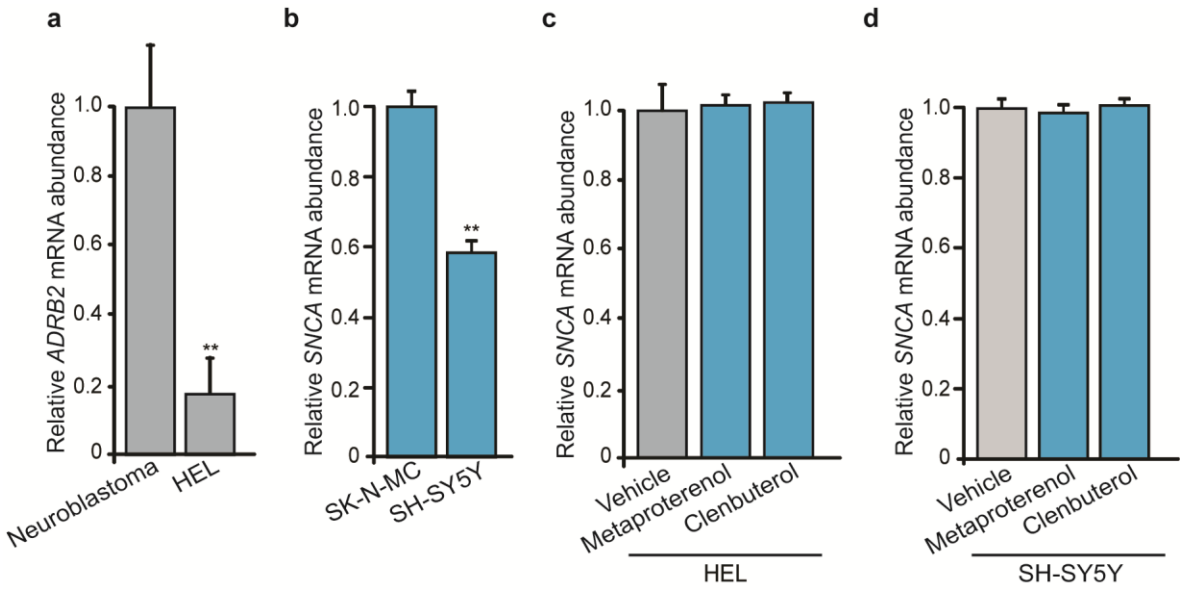

Figure S4. Effect of β_2AR agonists on *SNCA* expression is cellular context dependent. a) Relative abundance of endogenous *ADRB2* mRNA in HEL cells compared to neuroblastoma cells (as measured by qPCR). b) Relative abundance of endogenous *SNCA* mRNA in SH-SY5Y cells compared to SK-N-MC cells. c) Relative *SNCA* mRNA abundance in HEL cells treated with vehicle, metaproterenol (10 μM) or clenbuterol (20 μM) for 48 hours. d) Relative *SNCA* mRNA abundance in SH-SY5Y cells treated with vehicle, metaproterenol (10 μM) or clenbuterol (20 μM) for 48 hours. ***P*< 0.005, two-tailed Student's t-test; *n* = 6-8, means ± SEM are shown.

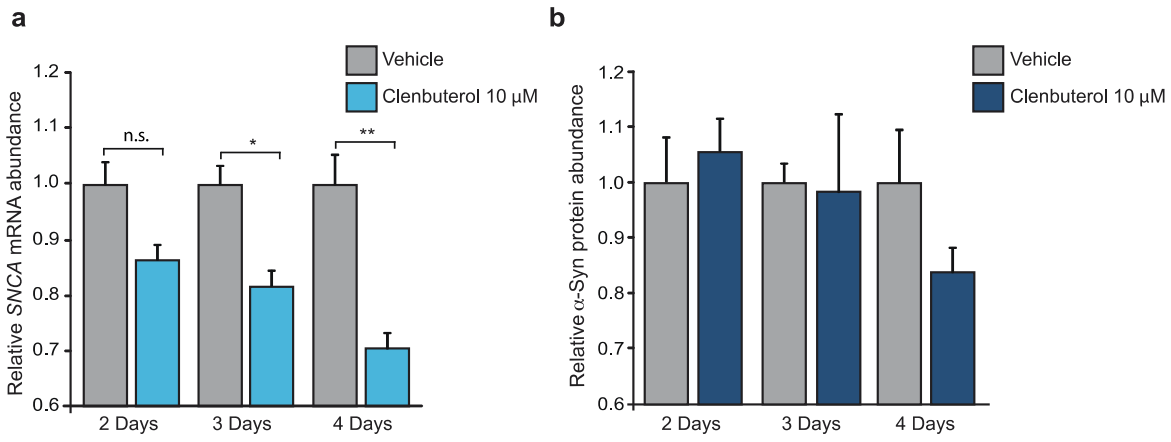

Figure S5. **β2AR agonists influenced *SNCA* expression in a time-dependent manner in neuroblastoma cells.** *P* values were < 0.05 by ANOVA for the overall interaction of drug x days of treatment on *SNCA* mRNA (a) and α-Syn protein (b) expression, respectively. **P*<0.05 and ***P*<0.005 for Tukey's post-hoc tests of contrasts at specific days; *n* = 6-8 per group, means ± SEM are shown.

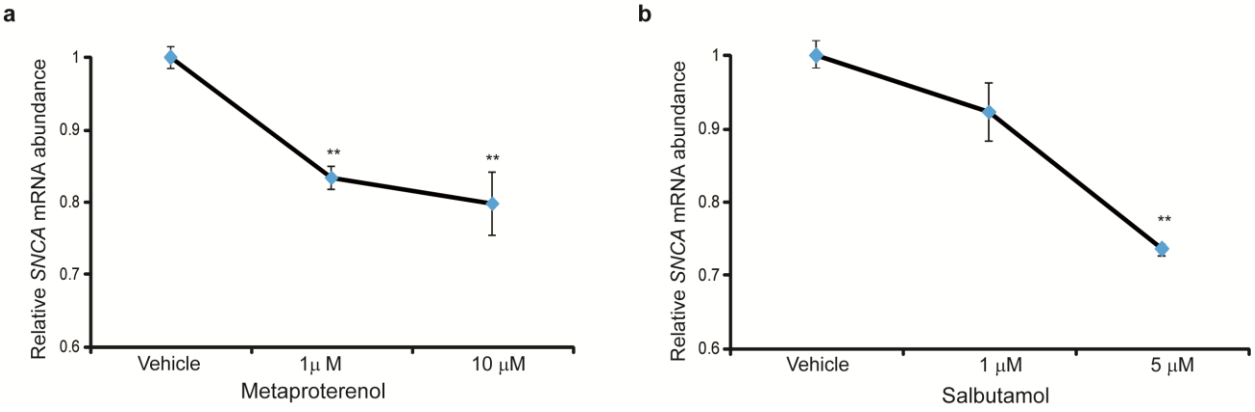

Figure S6. β_2AR agonists reduce *SNCA* expression in a dose-dependent manner. Relative *SNCA* mRNA abundance (as measured by qPCR) evaluated after 48 hours in SK-N-MC cells treated with the indicated doses of metaproterenol or salbutamol. ***P*< 0.005, ANOVA with Tukey's; *n* = 4-6, means ± SEM are shown.

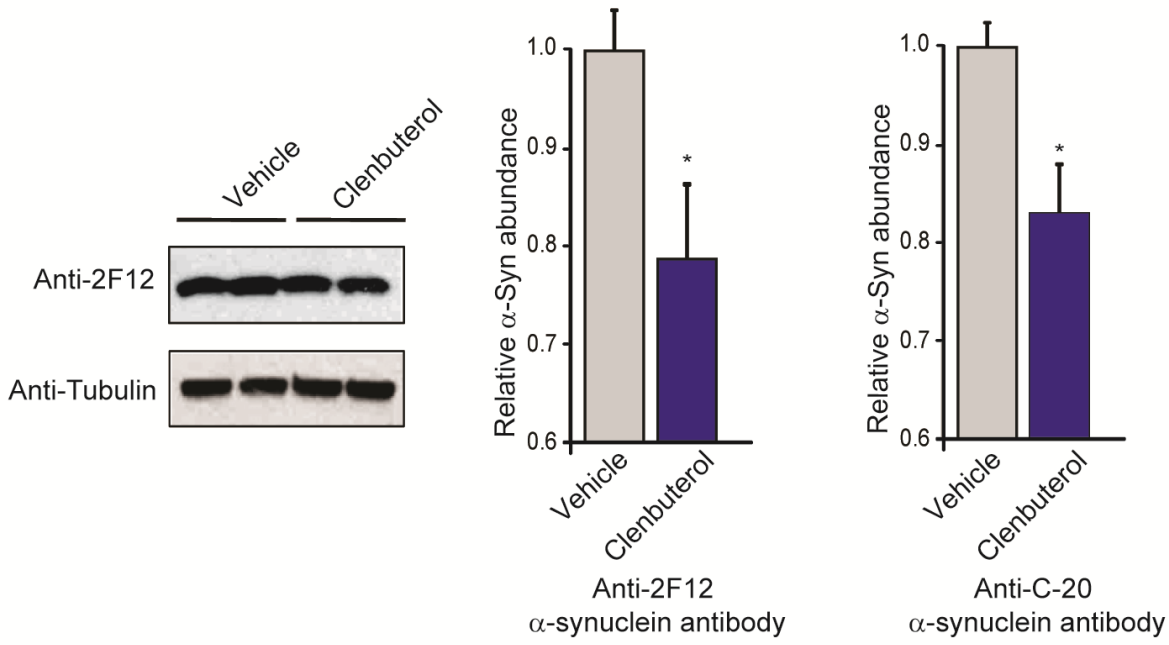

Figure S7. β_2AR agonist treatment reduces endogenous α-synuclein protein levels in the PD-vulnerable substantia nigra of mice as assayed by Western blot with two distinct anti-α-synuclein antibodies. Wild type C57BL/6J mice were treated with clenbuterol or vehicle (intraperitoneally) at 10 mg/kg body weight for 24 hours. a) A representative Western blot for endogenous α-synuclein protein abundance in substantia nigra homogenates of clenbuterol- and vehicle-treated mice is shown. b) Densitometric analysis showed a reduction of anti-α-synuclein immunosignal in clenbuterol treated mice relative to vehicle-treated mice (*n* =10 mice per group) using the anti-α-synuclein antibody 2F12. c) Similar results were obtained using the anti-α-synuclein antibody C-20. Anti-α-tubulin immunostaining was used to adjust for protein loading. **P*< 0.05, two-tailed Student's t-test; means ± SEM are shown.

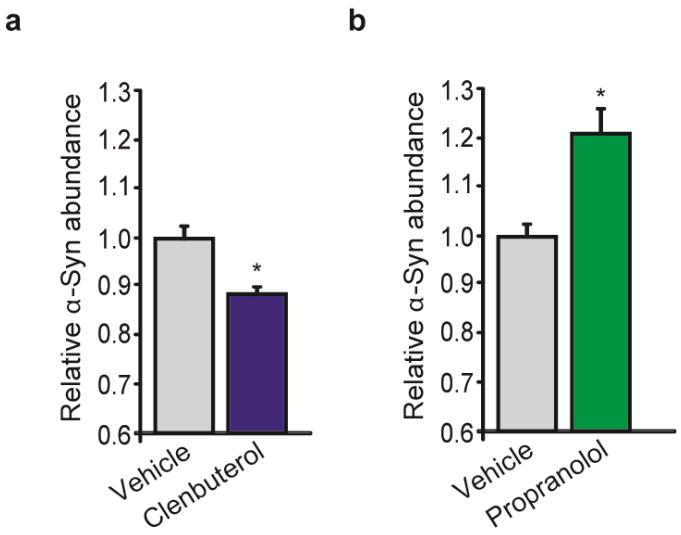

Figure S8. A second ELISA using distinct antibodies confirms that β_2AR agonist treatment reduces α-synuclein protein levels, while β_2AR antagonist treatment increases α-synuclein protein levels. Relative α-Syn protein abundance evaluated by a second ELISA using the anti-α-synuclein antibody Syn-1 as detection antibody and the anti-α-synuclein antibody 1H9 as capture antibody. Relative α-Syn protein abundance was evaluated after four days treatment. a) Neuroblastoma cells were treated with vehicle or clenbuterol (10 μM) for four days. b) Neuroblastoma cells were treated with vehicle or propranolol (100 μM) for four days. **P*< 0.05, two-tailed Student's t-test; *n* = 6, means ± SEM are shown.

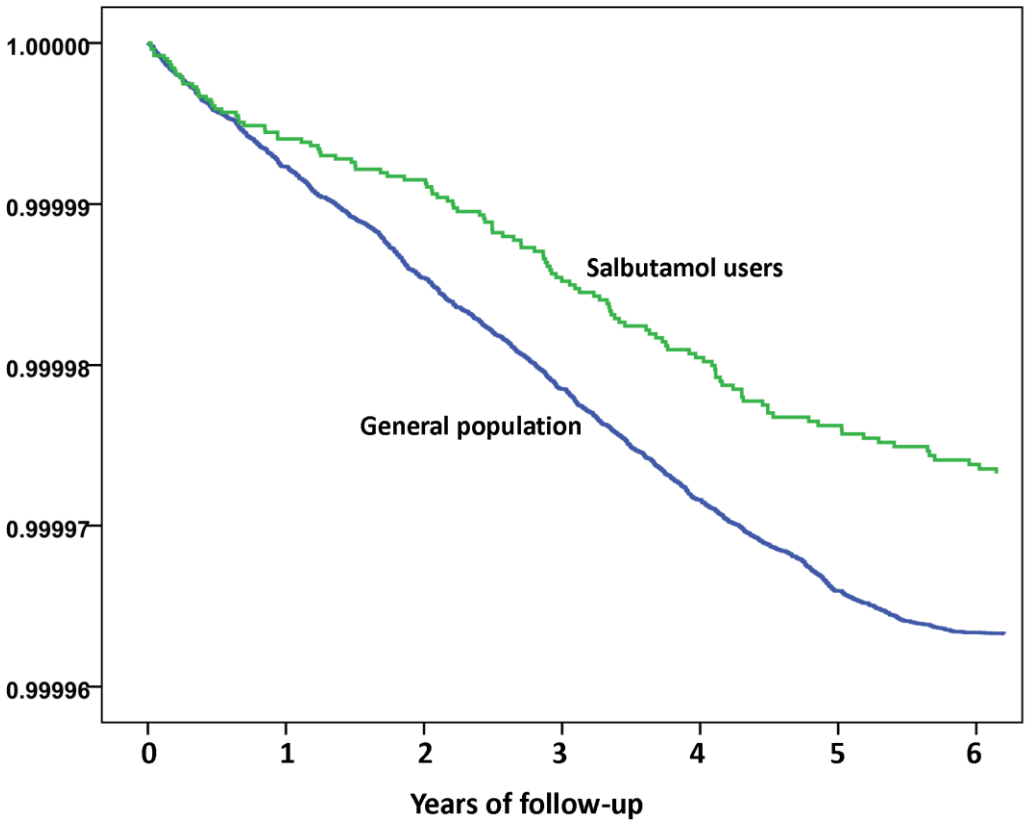

Figure S9. Salbutamol is associated with a significantly decreased risk for PD. Proportion of individuals not having developed Parkinson's disease during 2008-2014 among persons on salbutamol (during 2004-2007; *n* = 295,774) compared to the general population of Norway. Cox's proportional hazard regression model adjusted for age, sex and level of education.

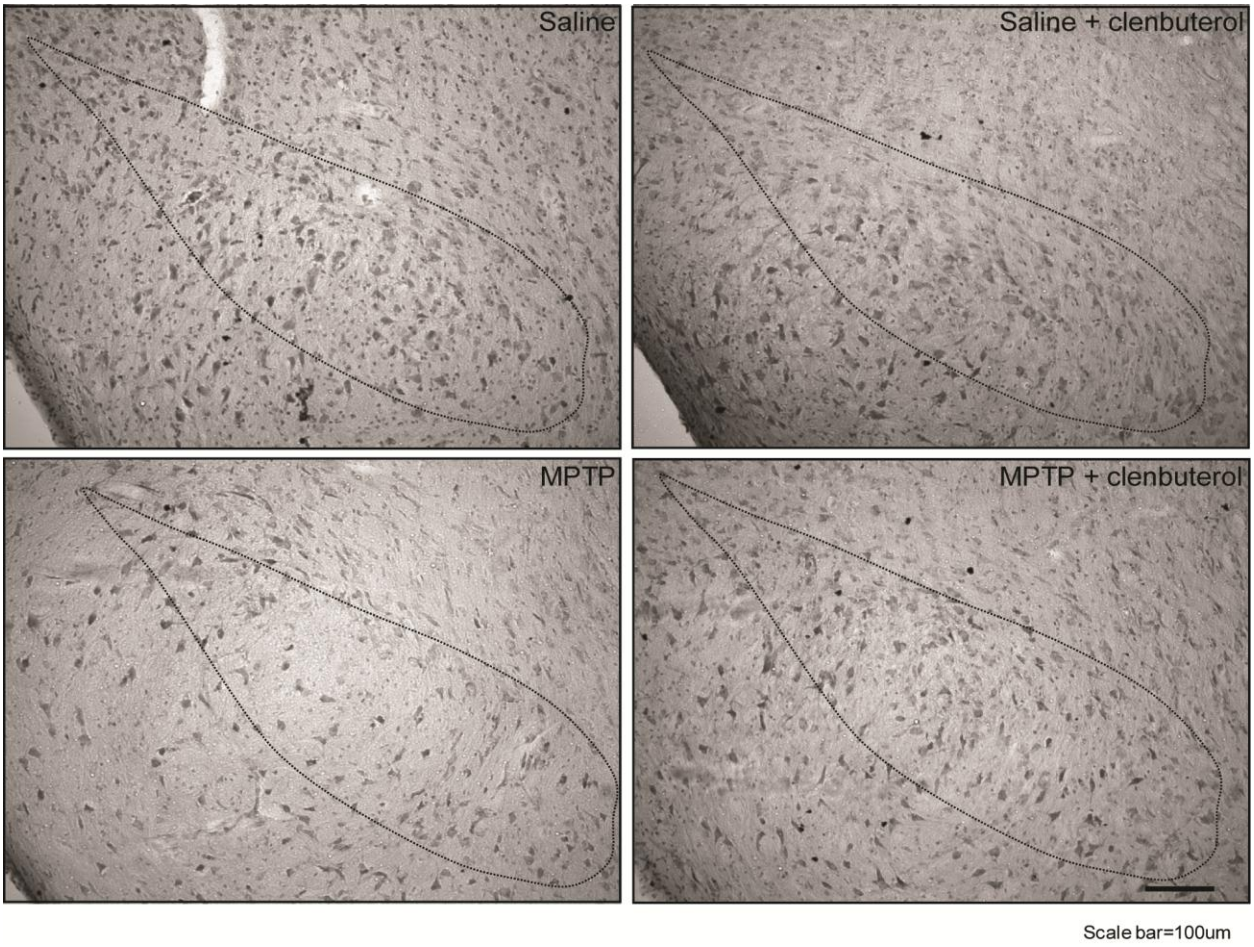

Figure S10. Clenbuterol treatment blocked MPTP-induced loss of cresyl violet positive cells in the substantia nigra pars compacta of wild-type C57bl/6 mice. Representative images illustrating cresyl violet positive cells in the SNpc region of C57bl/6 mice. MPTP induced loss of cresyl violet positive cells compared to vehicle and clenbuterol; whereas clenbuterol prevented MPTP induced-cell loss of cresyl violet positive cells (MPTP + clenbuterol). Scale bar: 100 μm.

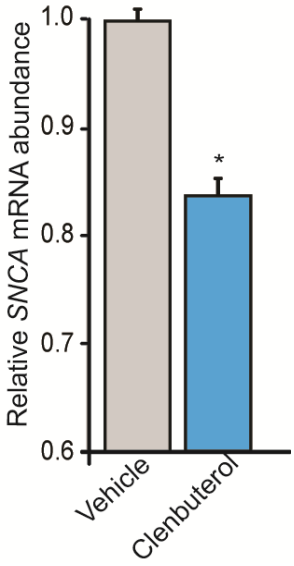

Figure S11. Clenbuterol treatment lowers *SNCA* expression levels in human iPSC-derived neurons of a patient carrying the locus triplication. Clenbuterol treatment significantly reduced endogenous *SNCA* mRNA expression compared to vehicle in *iPSC*-derived neurons cultured for 8 weeks followed by treatment with clenbuterol (20 μM) for three days. **P*< 0.05, two-tailed Student's t-test; *n* = 3, means ± SEM.

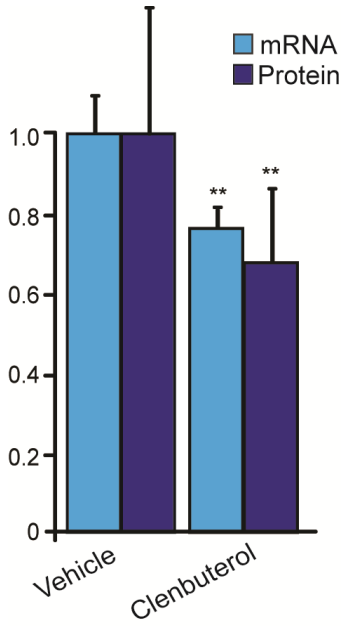

Figure S12. Prolonged β_2AR agonist treatment for 14 days also resulted in repressing *SNCA* expression. Relative *SNCA* mRNA and protein abundance evaluated by qPCR and ELISA, respectively, after 14 days of treatment with 10 μM clenbuterol in SK-N-MC cells. ***P*< 0.005, two-tailed Student's t-test; $n = 6$, means ± SEM.

4. Supplementary Tables

Table S1: Rate ratio (RR) of Parkinson's disease according to use of salbutamol and corticosteroids in a complete 11 years follow-up of the total Norwegian population.

				RR (95% CI)	
	Users	Cases	Person-years	Multivariate adjusted[a]	Multivariate adjusted[b]
Salbutamol					
Never user	4,066,119	4,398	36,700,554	1 (ref)	1 (ref)
Ever user	619,863	236	3,135,956	0.66 (0.58-0.76)	0.67 (0.59-0.77)
Corticosteroids					
Never user	4,412,626	4,480	38,262,872	1 (ref)	1 (ref)
Ever user	273,356	154	1,573,638	0.84 (0.71-0.98)	0.95 (0.80-1.12)

[a] Adjusted for age in 5 year periods, sex and level of education.
[b] Adjusted for age in 5 year periods, sex, level of education and salbutamol and corticosteroids simultaneously.
RR: Rate ratio; PD: Parkinson's disease; CI: Confidence interval

Table S2. Probes/ Primer information

	Gene (Human)	TaqMan assay
1.	*SNCA*	Hs00240906_m1
2.	*UBC*	Hs00824723_m1
3.	*RPL13*	Hs00761672_s1
4.	*GUSB*	Hs99999908_m1
5.	*NEFL*	Hs00196245_m1
6.	*ADRB2*	Hs00240532_s1

	Gene (Mouse)	TaqMan assay
1.	*Snca*	Mm01188700_m1
2.	*Ubc*	Mm01198158_m1
3.	*Rpl13*	Mm02342645_g1
4.	*Actb*	Mm00607939_s1

	Gene (Rat)	TaqMan assay
1.	*Snca*	Rn00569821_m1
2.	*Ubc*	Rn01499642_m1
3.	*Rpl13*	Rn00821258_g1
4.	*Actb*	Rn00667869_m1

	Gene	Target sequence for NanoString probes
1.	*SNCA*	GGGCAAGGTATGGCTGTGTACGTTTTGTGTTACATTTATAAGC TGGTGAGATTACGGTTCATTTTCATGTGAGGCCTGGAGGCAGG AGCAAGATACTTAC
2.	*RPL13*	GGGCCTGGGATGGGGCTTCACTGCTGTGACTTCCTCCTGCCAG GGGATTTGGGGCTTTCTTGAAAGACAGTCCAAGCCCTGGATAA TGCTTTACTTTCTG
3.	*UBC*	TGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCT CGAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAA GATCCAAGACAAGGA
4.	*GUSB*	CGGTCGTGATGTGGTCTGTGGCCAACGAGCCTGCGTCCCACCT AGAATCTGCTGGCTACTACTTGAAGATGGTGATCGCTCACACC AAATCCTTGGACCC

	CHIP assay	Forward Primer	Reverse Primer
1.	Promoter (insert 1)	ACTTAACGTGAGGCGCAAAA	CTATCTCGGATGGGGATGG
2.	Enhancer (insert 2)	GACAAGGGAAGGTGGATGAA	GGGGATGCTTTTACCAGTGA
3.	Enhancer (insert 3)	CACAATTGGCCCTGGATTAG	TCCCTGTTTCCTCTTTGTGG